Remarks

Claims 36, 39, 41-43, 46-49 and 57-70 are currently pending. Claims 36, 61, 64, and 65 have been amended and claims 41-43, 57-60, and 66-70 have been canceled. Support for the claim amendments may be found throughout the specification, including the claims as originally filed. In particular, support for the amendments to claims 36, 61, 64 and 65 may be found, for example, on page 30, lines 3-8, page 26, lines 13-22, page 27, lines 10-21, page 114, lines 4-12. No new matter has been added.

Cancellation and/or amendment of claims should in no way be construed as an acquiescence to any of the Examiner's rejections. Cancellation and/or amendments to the claims are being made solely to expedite prosecution of the present application and do not, and are not intended to, narrow the claims in any way. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

Specification

Applicants thank the Examiner for pointing out the pages in the specification which indicate that βTrCP is a human homolog of Cdc4p. As explained in the Applicant's Response of April 7, 2004, βTrCP and Cdc4p are not the yeast and human homolog of the one and the same protein. Applicants have amended the specification. This amendment is believed to obviate the objection. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claim Objections

Claims 36, 39, 41-43, 46-49, 62, 65-67, 69 and 70 were objected to for reciting non-elected inventions, SEQ ID NOs; 2, 6, 8, 10 and 12 encoded by SEQ ID NOs: 1, 5, 7, 9 and 11. Claims 36, 42-43, 65-67 and 69-70 have been amended. This amendment is believed to obviate the objection. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claim 41 was objected to under 37 C.F.R. 1.75(c) as being of improper dependent form for failing to limit the subject matter of a previous claim. Solely to expedite prosecution, Claim 41 has been canceled, thereby rendering this objection moot.

Claim 42 was objected to under 37 C.F.R. 1.75(c) as being of improper dependent form for failing to limit the subject matter of a previous claim. Solely to expedite prosecution, Claim 42 has been canceled, thereby rendering this objection moot.

Rejection of Claims 36, 39, 41-43, 46-49, 60, 62 and 63 under 35 U.S.C. § 112, first paragraph

Claims 36, 39, 41-43, 46-49, 60, 62 and 63 were rejected under 35 U.S.C. § 112, first paragraph for reasons of written description. The Examiner states that "[w]hile the specification provides support for a nucleotide sequence that is at least 90% or 95% identical to a nucleic acid sequence shown in one of the sequence listings" that she is "unable to locate adequate support in the specification for at least 90% or 95% identity to a fragment of a specific nucleotide sequence, including SEQ ID NO:3."

Applicants respectfully disagree, however, in an effort to expedite prosecution of the application, claim 36 has been amended and claim 42 has been canceled. Support for the amendment to Claim 36 can be found through out the specification, for example, on page 30, lines 3-8, page 26, lines 13-22, page 27, lines 10-21, page 114, lines 4-12. This amendment is believed to obviate the objection. No new matter was introduced by this amendment. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

The Examiner further states, with regard to claim 60, that she is unable to find support for a chimeric sequence comprising F-box encoded by a fragment of SEQ ID NO: 3 (i.e. 100% identical and further comprising a WD domain encoded by a nucleotide sequence that is at least 95% identical to SEQ ID NO: 3. In a effort to expedite prosecution, claim 60 has been canceled, thereby rendering this rejection moot.

Rejection of Claims 36, 39, 41-43, 46-49 and 57-64 under 35 U.S.C. § 112, first paragraph

Claims 36, 39, 41-43, 46-49 and 57-64 were rejected under 35 U.S.C. § 112, first paragraph, for reasons of written description. The Examiner states that claims 36 and 42 and any claims that depend thereon do not identify the structure of the claimed F-box in terms of its length and sequence. Claim 36 has been amended and the amendments are believed to obviate the rejection. No new matter was introduced by this amendment. Claim 42 has been canceled, thereby rendering this rejection moot. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

The Examiner also asserts that the genus of the target polypeptide interaction domain encompasses polypeptides of greatly variable structure and function. Applicants respectfully traverse the rejection.

Although the Examiner is correct that the instant specification only discloses certain interacting domains for particular proteins, Applicants maintain that there is appropriate written description of the genus based on publicly available information. In particular, information regarding binding partners for virtually any protein can be obtained from protein-protein interaction databases. In addition, as further described below, the instant specification provides guidance for identifying an interacting domain for any particular protein. Accordingly, Applicants request that this rejection be withdrawn.

Claims 36, 39, 41-43, 46-49 and 57-70 were further rejected under 35 U.S.C. § 112, first paragraph, for reasons of enablement. The Examiner asserts that the specification "while being enabling for a method for targeting a target polypeptide using hybrid polypeptides comprising an F-box of Cdc4 or βTrCP and a known target polypeptide interaction domain, such as LTP and E7N, in yeast and human cells, respectively, does not reasonably provide enablement for a method of used of a hybrid polypeptide comprising any F-box and any target polypeptide interaction domain for targeting for ubiquitin proteolysis in any eukaryotic cell." Applicants respectfully traverse this rejection.

The specification describes that expressing a hybrid protein comprising an F-box and a target polypeptide interaction domain that binds to a target polypeptide in a cell results in target degradation of the target polypeptide. Without wanting to be limited to a particular mechanism of action, it is believed that the hybrid protein comprising an F-box recruits the target polypeptide interacting with the hybrid protein to the Skp1/Cul1/F-box (SCF) ubiquitin ligase complex and is targeted for ubiquitin-dependent proteolysis. Further, the specification describes that F-boxes from different proteins are high conserved and thus have a similar structure. Given the high conservation, an F-box from one protein from one eukaryotic species is believed to target proteins for degradation in cells of the same eukaryotic species, as well as cells from other eukaryotic species.

The specification further describes that target polypeptide interaction domains, if not known in the literature, can readily be isolated (see, e.g., pages 33 to 58). Among the recited methods are the yeast two-hybrid or interaction trap, the yeast cytoplasmic two-hybrid, the mammalian two-hybrid or interaction trap, the far western, phage display, protein trap + nucleic acid snag, biomolecular interaction analysis and peptide matrix arrays. Table I on page 36 provides a summary of the general methods, along with references, that may be used to clone interacting proteins and pages 37-58 provide additional guidance on these methods. Based on the guidance provided in the specification and the advanced level of the knowledge in the art, Applicants respectfully submit that a person skilled in the art would readily be able to identify and isolate a polypeptide interaction domain that could be used to construct a hybrid polypeptide of the present invention.

The specification provides working examples of fusion proteins comprising an F-box from two different proteins: pCdc4 and βTrCP. Regarding target proteins, the specification provides working examples of three different target proteins: pRb, p107 and the viral protein E2. As discussed in Applicant's previous response, since filing of the application, the Applicants have also successfully targeted other cellular proteins, e.g., p130, a retinoblastoma protein family member (Zhang et al. (2003) *PNAS* 100:14127) and β-catenin (Cong et al. (2003) *BMC Molecular Biology* 4:10).

Also as discussed in the Applicant's previous response, since the filing of the application, several articles have been published describing a similar system targeting a target protein for ubiquitin-mediated degradation by the SCF ubiquitin ligase complex. Liu et al. ((2004) *BBRC* 313:1023) and Su et al. ((2003) *PNAS* 100:12729) both describe the targeted degradation of β-catenin using different hybrid proteins.

Further, two additional papers have been published which use the system described in the instant application for targeting a target protein for ubiquitin-mediated degradation by the SCF ubiquitin ligase complex. Specifically, Cohen et al. targets c-Myc for ubiquitin-mediated degradation by the SCF ubiquitin ligase complex using a hybrid polypeptide of βTrCP fused with the helix-loop-helix/leucine zipper region of Max, a known interaction domain of c-myc (2004 *BMC Developmental Biology* 4:4). Chen et al. used a chimeric construct of βTrCP fused to the LFG peptide to target cdk2 and its binding substrate, cyclin A, for ubiquitin-mediated degradation (2004 *Cancer Res.* 64:3949). In addition, both Cohen et al., *supra*, and Chen et al., *supra*, shows that the hybrid polypeptides of the instant application can be used for targeting a target protein for ubiquitin-mediated degradation in animal models. Accordingly, the specification enables a person of skill in the art to make and use the invention commensurate with the scope of the claims.

In applying the Wands factors, it is evident from the above discussion that the level of skill in this art was very high at the time the application was filed, and the experimental techniques needed to practice the invention were well known and exemplified in the specification as filed. Accordingly, Applicants respectfully submit that armed with the teachings of the specification and the contemporary knowledge in the art, the skilled artisan would be able to practice the claimed methods without further undue experimentation.

The Examiner's specific arguments are believed to be addressed within the above paragraphs, as well as in the Applicant's previous responses. Thus, reconsideration and withdrawal of this rejection is respectfully requested.

Rejection of Claims 65-70 under 35 U.S.C. § 112, first paragraph

Claims 65-70 were rejected under 35 U.S.C. § 112, first paragraph, for reasons of enablement. Applicants respectfully traverse the rejection. However, in an effort to expedite prosecution, Applicants have amended claim 65. This amendment is believed to obviate the rejection. No new matter has been added. Further, claims 66-70 have canceled claims 66-70, thereby rendering the rejection moot. Reconsideration and withdrawal of the rejection is respectfully requested.

Rejection of Claims 36, 39, 41-43, 46-49 and 57-70 under 35 U.S.C. § 112, second paragraph

Claims 36, 39, 41-43, 46-49 and 57-70 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

In particular, claim 36 was rejected for reciting a hybrid polypeptide comprising an "F-box" and then subsequently reciting "wherein the F-box recruits the hybrid polypeptide to a Skp1/Cul1/F-box protein (SCF)." The term "Skp1/Cul1/F-box protein (SCF) ubiquitin ligase complex" refers to a specific protein complex, which plays a role in providing the specificity of substrate recognition for ubiquitin dependent proteolysis. The term "Skp1/Cul1/F-box protein (SCF) ubiquitin ligase complex," is also known as the E3 complex or the E3 ubiquitin protein ligase complex as defined on page 16, lines 17-23, of the instant application. The SCF complex consists of core protein components (i.e., Skp1, Cul1 or Cdc53) as well as an F-box protein. The F-box protein in the complex is the same F-box protein recited in the first part of the claim.

Claims 36, 42 and 43 were rejected as allegedly being unclear as to what sequences were being claimed. Claim 36 has been amended and claims 42 and 43 have been canceled. The amendment to claim 36 and the cancellation of claims 42 and 43 are believed to obviate the rejection. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Claim 39 recites the phrase "proteolysis is by the proteasome," which was indicated as missing by the Examiner. This rejection is respectfully traversed. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 41 was rejected as allegedly being unclear which sequences other than SEQ ID NO: 2, 4, 6, 8, 19, 12 are encompassed by the terms Cdc4p, beta TrCp, Grr1p, Met30p, Pop2p, and FWD1, respectively. Applicants respectfully traverse the rejection, however, in an effort to expedite prosecution, claim 41 has been canceled.

Claim 49 was rejected as allegedly unclear as to which sequences are encompassed by the recited terms. Applicants respectfully traverse the rejection. Each of the recited terms refer to known proteins, such that one of ordinary skill in the art would know how to determine the sequence of each protein using Genbank or another protein database. Further, the recited terms are exemplary sequences that may be targeted using the invention. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Claims 65 and 70 were rejected as allegedly unclear as to which sequences compose an "SCF ubiquitin ligase complex." Applicants respectfully traverse the rejection. Specification defines an SCF ubiquitin complex on page 16, lines 17-23, and provides support for exemplary SCF subunit-encoding genes on page 177, lines 11-15. Additional sequences are found on pages 155-156 for βTrCP. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Rejection of Claims 36, 39, 41-43, 46, 47, 49, 65-67, 69 and 70 under 35 U.S.C. § 102(a)

Claims 36, 39, 41-43, 46, 47, 49, 65-67, 69 and 70 are rejected under 35 U.S.C. § 102(a) as being anticipated by Kumar et al. The Examiner asserts that Kumar et al. teaches "chimeric polypeptides wherein F-box of SCON2 proteins were replaced with foreign F-box containing proteins such as Cdc4p and Met30p." The Examiner further asserts that Kumar et al. teaches a method of targeting a polypeptide comprising an F-box and a target interaction domain for proteolysis in a eukaryotic cell.

Applicants respectfully disagree. Kumar et al. teaches the role of SCON2, an F-box protein, in sulfur regulation in *Neurospora crassa*. Using a chimeric construct, Kumar et al. shows that when the F-box motif of SCON2 is replaced with the F-box motif from Cdc4p and Met30p that there is partial function of the foreign F-box in SCON2 in regulating the *Neurospora* sulfur system. Kumar et al. speculates that "the ability of these swapped domains to at least partially function within SCON2 suggests a common underlying mechanism of action, possibly involving F-box-mediated proteolysis." Kumar et al. does not teach that SCON2 functions through a proteolytic process.

Further, Kumar et al. does not teach any of the claim limitations of the instant application. In particular, Kumar et al. does not teach human βTrCP or SEQ ID NO:4, which are the subject of the instant application. Further, Kumar et al. does not teach how to target a polypeptide interaction domain that binds to a target polypeptide in a eukaryotic cell such that the target polypeptide is degraded via ubiquitin-dependent proteolysis. Therefore, Kumar et al. does not anticipate the instant application. Applicants respectfully request reconsideration and withdrawal of these rejections based on the disclosure of Kumar et al.

Conclusion

In view of the above remarks and the amendments to the claims, it is believed that this application is in condition for allowance. If a telephone conversation with Applicant's Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1000.

If any additional fees are due, the Commissioner is hereby authorized to credit any overpayment or charge any deficiencies to Deposit Account Number **06-1448, Reference HMV-043.01**.

Respectfully submitted,

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